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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The purpose of this project is to search for mutations that affect breast cancer by screening the progeny of mice that were injected with ethylnitrosourea (ENU). We began the project by treating BALB/c males with ENU, and then mating them with MN-10 partners. MN-10 is a strain which carries the c-neu oncogene under the control of an MMTV promoter, on the BALB/c background. Although some of these matings were successful, we found this strain to be unusually sensitive to ENU. We therefore adopted an alternative strategy, and utilized a strain which carries a similar mutation, but which has the FVB/NJ background. Our experience with this strain, known as FVB-TgN(MMTVneu)202Mul, was vastly more successful. Most of the treated males recovered their fertility rather quickly after ENU treatment, and produced large numbers of progeny. The female progeny of these mice are now being observed for the occurrence of breast cancer. Although we have not yet seen any evidence of a mutation that affects breast cancer, we believe that we have a mutation that affects the spleen and lower G.I. tract. The overall significance of this is that if we can identify mutations in mice that affect susceptibility to breast cancer, we can identify the corresponding human gene.

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Introduction

Our strategy for this project is based upon the idea that cancer is the end result of a series of genetic changes, mostly involving somatic mutations in the developing tumor. We proposed that we would be able to identify some of the genes that become mutated in breast cancer if we would start with a transgenic line of mice that already carries one mutation known to be involved in breast cancer, *c-neu*, and mate it with partners who have been treated with N-ethyl-N-nitrosourea (ENU) to induce mutations. Progeny of these matings which carry both the *c-neu* oncogene and a mutant gene that can interact with it to enhance mammary tumors will be especially sensitive to breast cancer development. Any gene that inhibits breast cancer development would cause a lower degree of susceptibility than what is observed in control mice that carry only the *c-neu* oncogene. It is our proposal to map any mutant gene of this type with respect to known microsatellite markers, by using the polymerase chain reaction (PCR). After identifying a sufficiently precise map position, it should be possible to clone the gene by positional cloning, and to identify its human counterpart. This final goal is beyond the scope of the present project, but because it will eventually be feasible, this project has a considerable degree of clinical relevance.

Body

We have now completed the second year of our Idea Grant project. We initially proposed to study the progeny of 180 ENU-treated males, and more than that number have now been injected with ENU (189). The female progeny must undergo pregnancy and lactation in order for the MMTV-*neu* oncogene to be activated, and that is the phase that we are now working on. Therefore, we have completed Task 1. To our disappointment, none of the female progeny of the treated males have developed mammary tumors yet, but the period of observation has not yet reached 6 months for most of the lines. Furthermore, most of the observations so far have been limited to females who possessed only one prospective mutant allele (i.e. searching for a mutant gene that behaves as a dominant). We have intercrossed the progeny of many of the lines, and now have available second-generation mice that should be homozygous for mutant genes. We have observed three tumors so far, but they have all been in control mice at an age of 6 months or greater.

We are continuing our observations on 46 lines of mice at present (i.e. descendents of 46 different treated males). Numerous other treated males are still in matings, but have not yet recovered their fertility. Some of the mice that were injected with the highest dose (100 mg/kg) never did regain fertility and eventually had to be euthanized. Ideally we would continue injecting additional males with ENU to substitute for those mice, but we are not doing that for two reasons. (a) More than 12 months is required from the time of injection to the scoring of breast cancer in female progeny, and we have only one year remaining in this project. (b) The size of our mouse colony is at the maximum that can be supported by the available funds. Therefore, we are

concentrating on scoring when breast cancer occurs in the females of the first and second generation descendents of the treated males.

Although we have not yet observed any mutant genes that affect tumor susceptibility, we believe we have obtained a mutant gene for a different phenotype. In the third line that we established, the progeny of the treated male were mated with one another, and among those progeny were four mice that had a "failure to thrive" phenotype. The mice were euthanized and autopsied, and there were no tumors present that could have accounted for these problems. The spleens had a very abnormal appearance in histological sections, and it looks as if a great deal of extra-medullary hematopoiesis is occurring. There were also abnormalities in the histological sections from the gastrointestinal tract. The defect is not segregating as a single gene trait with complete penetrance, so we believe that it is either determined as a single-gene recessive trait with incomplete penetrance, or by multiple genes. Although this is not the type of trait that we proposed to study, we are encouraged by the evidence that we apparently have isolated a mutant gene that affects a clinically significant trait.

Key Research Accomplishments

One accomplishment of the past year has been the characterization of the ENU susceptibility of two strains that have proved to be very different from each other. We have found the BALB/c substrain that we are using (from Taconic) to be very difficult to use in these experiments, as it is highly susceptible to the effects of ENU. Many treated mice die at dosage levels that are considered optimal for other strains (80 to100 mg/kg), and many of those that survive this treatment never regain their fertility. This differs from what had been reported previously with other substrains (Justice et al., 2000). On the other hand, we have found the FVB/NJ strain to be very well suited for this type of experiment, and we were able to make use of a line that carries the c-neu oncogene under the control of an MMTV promoter (Li et al., 1997). These mice were able to tolerate multiple doses of up to 80 mg of ENU/kg of body weight, and they regained their fertility much more rapidly than any of the BALB/c mice that we injected. Furthermore, they have a very high degree of fertility, so any interesting mutants that are produced in these mice can be propagated very easily.

A second accomplishment is the isolation of what we believe to be a new mutation (or mutations) that affect failure to thrive. These mice have a very abnormal appearance in histological sections of the spleen, and some abnormalities of the GI tract. We are currently attempting to characterize this more precisely.

Reportable Outcomes

We plan to notify our colleagues in other laboratories to make use of the FVB/NJ strain for experiments on ENU-induced mutagenesis. We have found them to tolerate the injections exceptionally well, and to recover their fertility much more rapidly than the

BALB/c strain. Because they have such a high degree of fertility, they will be ideally suited for the rapid propagation of interesting new mutants.

Our finding of an apparent mutation that affects failure to thrive is not yet reportable in the scientific literature, but after further characterization, we believe this will prove to be the most interesting observation that we made during the past year.

Conclusions

We have completed the injection phase of our project, and are now observing the progeny of the treated mice for possible mutant phenotypes. First-generation progeny are being observed for effects that are inherited as dominant traits, and second-generation progeny are being observed for susceptibility traits that are inherited as recessives. We believe that we have identified one mutant phenotype that involves failure to thrive, and is accompanied by an abnormal splenic and gastrointestinal histology. We have had our greatest success with ENU treatments when we used mice that have the FVB/NJ background.

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